

Amendments To the Claims

Claim 1 (Currently amended): A computer-based method of modeling a directed evolution protocol method comprising:
applying equilibrium thermodynamics to a plurality of sequences to determine statistics of hybridization; and
parameterizing an assembly algorithm using the statistics of hybridization;
applying the assembly algorithm to reassemble a plurality of sequences; and
providing a representation of the plurality of sequences.

Claim 2 (Cancelled)

Claim 3 (Currently amended): The method of claim 21 further comprising determining crossover allocation in the plurality of reassembled sequences.

Claim 4 (Original): The method of claim 3 wherein the step of determining crossover allocation includes estimating a fraction of the plurality of reassembled sequences containing a number of crossovers.

Claim 5 (Original): The method of claim 3 wherein the step of determining crossover allocation includes estimating a probability that a given nucleotide position in one of the plurality of reassembled sequences is a site of a crossover event.

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Claim 6 (Original): The method of claim 1 wherein the directed evolution protocol is DNA shuffling.

Claim 7 (Original): The method of claim 1 wherein the directed evolution protocol is SCRATCHY.

Claim 8 (Original): The method of claim 1 further comprising identifying a minimum number of required silent mutations to meet a DNA recombination objective.

Claim 9 (Original): The method of claim 1 wherein the step of applying equilibrium thermodynamics to determine statistics of hybridization includes:
modeling annealing events during reassembly as a network of reactions;
determining a predicted fraction of fragments that will anneal at a given temperature;
determining a predicted distribution of annealing for overlap lengths; and
determining a portion of annealing events predicted to involve mismatches.

Claim 10 (Original): The method of claim 1 wherein the assembly algorithm excludes silent crossovers.

Claim 11 (Currently amended): ~~A~~An *in vitro* method of providing an isolated nucleic acid molecule derived from a directed evolution experiment comprising:

providing a nucleotide sequence ~~having capable of encoding an amino acid sequence;~~ wherein
the nucleotide sequence isolated at least in part through a directed evolution experiment;
and
the directed evolution experiment selected at least in part by applying equilibrium
thermodynamics to a plurality of sequences to determine statistics of hybridization and
parameterizing an assembly algorithm using the statistics of hybridization.

Claim 12 (Previously amended): The method of claim 11 wherein a vector comprises the
nucleic acid molecule.

Claim 13 (Previously amended): The method of claim 12 wherein a host cell contains the
vector.

Claim 14 (Cancelled)

Claim 15 (Currently amended): A system for modeling a directed evolution protocol
comprising:
a plurality of sequences; and
an article of software for determining statistics of hybridization of the plurality of sequences to
parameterize an assembly algorithm by applying equilibrium thermodynamics to the plurality
of sequences in order to model the directed evolution protocol.